# PRELIMINARY PROPOSAL FOR FY 2005 FUNDING

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| Title: | Assessing critical assumptions associated with survival estimates of subyearling Chinook salmon using radio-telemetry, 2005 |
| Study Code: | SPE-04-NEW |
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PROJECT SUMMARY

## Introduction

This proposal addresses critical assumptions for estimating survival probabilities of subyearling Chinook salmon. Research addressing these assumptions began in the summer of 2004 and consisted of four objectives that were identified in the one-page statement of research needs. Research for Objectives 1 and 3 will be completed during 2004. This proposal describes research for Objectives 2 and 4 that we propose for continuation through fiscal year 2005.

We believe research activities proposed for summer of 2005 are justified based the following information.

1) Bench tests conducted in 2004 indicate that the antenna length of radio tags can be reduced significantly, perhaps as much as 30%, without affecting the detectability of the tag. A shorter antenna could benefit the fish by reducing drag and minimize the potential effects that the antenna length has on performance parameters like burst swimming, orientation, and buoyancy.

2) It may be feasible to switch to antenna material that is about 50% of the weight and diameter of the antenna currently used. This, together with advancements being made by radio tag vendors, could reduce the weight of the currently used tag by 40% from 0.85 g to 0.5g. A significant reduction in weight could minimize the potential effects of the tag on performance parameters like burst swimming, orientation, and buoyancy.

3) A tag weighing 0.5g could allow researchers to tag fish as small as 95 mm. The ability to tag smaller fish means that radio telemetry studies will represent a greater portion of the population. For example, subyearling Chinook salmon >95 mm represented over 80% of the population passing McNary Dam between 2000 and 2004. The current tag (weighting 0.85g) used fish that represented only 15% of the population passing McNary Dam between 2000 and 2004.

4) Because a smaller tag can be implanted in a greater proportion of the population, less fish will need to be handled at the collection facilities to obtain the desired sample size for radio telemetry studies. For example, if a 0.5g tag were used during 2004 instead of the 0.85g tag, the John Day Dam Fish Collection Facility would have had to handle only 18% of the fish that were handled in 2004 to obtain the sample size needed to meet survival and FPE objectives.

5) In 2005, we propose to conduct performance tests during the outmigration season at one of the main stem fish collection facilities. Extension of the laboratory tests to the field during the summer of 2005 will serve as quality control for survival estimates of relatively small Chinook salmon that we have not tagged in past studies. We will use run-of-the-river fish that will be more representative of the fish being used for survival estimates. Furthermore, the thermal history of fish migrating in river will be more representative of fish used for survival studies.

6) Experiments conducted in 2004 incorporated a tag that was only partially neutrally buoyant because we were concerned that the increased volume of this tag may limit implantation into smaller individuals. If these experiments determine that the partially neutrally buoyant tag has less of an effect on fish than standard tags, then we propose to extend the experiments. In 2005 we propose to conduct experiments in the field to determine whether it is feasible to implant fish with larger volume tags that are more neutrally buoyant than the tags tested in 2004.

7) As was the case in 2004, experiments proposed for 2005 will explicitly incorporate fish size as a factor. This will allow us to determine the threshold where transmitters begin to affect the performance of tagged subyearling Chinook salmon. Because there has been no consistent guidance on maximum tag ratios or the transmitter technology with the least effect on fish, the trend has been to conduct a laboratory study with smaller fish each time a new transmitter is developed. In contrast, our goal is to provide general criteria that can be applied to different sizes and types of transmitters as they are developed. This information should reduce the need to conduct another laboratory study each time a smaller transmitter of a particular technology is developed.

## Research Goals

The goal of this research is to address critical uncertainties for studies that use radio-tagged subyearling Chinook salmon to evaluate the effects of dam operations occurring in summer.

## Study Objectives

**Objective 1:** Determine whether the effects of spill operations on the estimated survival of subyearling Chinook salmon changes through time (e.g., 20 June - 20 July; retrospective analysis of available spill studies) and whether there is a differential effect of spill treatments on subyearling Chinook salmon survival of varying sizes.

*Note: Objective 1 is complete and will not be repeated or expanded on for 2005.*

**Objective 2**. Determine the effects of radio-tagging subyearling Chinook salmon at elevated water temperatures.

*Note: Objective 2 will be will be continued in the field for 2005.*

**Objective 3.** Determine the effects of altering transmitter antenna length and material on transmitter power and reception.

*Note: Objective 3 is complete and will not be repeated or expanded on for 2005.*

**Objective 4**. Identify the transmitter technology that minimizes negative effects on subyearling Chinook salmon used in telemetry studies.

*Note: Objective 4 will be continued in the field for 2005.*

## Relevance to the Biological Opinion

This study addresses the 2000 Biological Opinion reasonable and Prudent Action number 82 (NMFS 2001).

## PROJECT DESCRIPTION

## Background and Justification

Spill has long been used as mitigation at hydroelectric projects to increase the survival of juvenile salmonids. In December 1988, a 10-year spill program was developed for implementation at projects that were not equipped with adequate bypass systems to achieve fish passage efficiency goals (Fish Spill Memorandum of Agreement). Continued declines in salmonid stocks prompted the development of a more aggressive spill management plan under the NMFS Biological Opinion (NMFS 2001). However, the increasingly aggressive spill scenarios were required to conform to the water quality criteria (i.e., total dissolved gas levels) established in the Clean Water Act (*http://www.epa.gov/region5/water/cwa.htm*). Consequently, the current NMFS BIOP for Bonneville Dam recommends 24 h spill with 75 kcfs during the day and total dissolved gas cap during night up to 125% in the tailrace of the dam to aid fish passage efficiency and survival.

Benefits of spill (decreased mortality, migration delay, and predation risk) have been documented and accepted in the scientific community (Whitney et al. 1997; Muir et al. 2001; Giorgi et al. 2002; Dehart 2003). Previous studies have found that passage survival for juvenile salmonids was generally highest for spillways, followed by bypass systems and then turbines (Schoeneman et al. 1961; Whitney et al. 1997; Muir et al. 2001). Whitney et al. (1997) reviewed 13 (3 steelhead and 10 salmon) estimates of spill mortality and concluded that 0 to 2% is the most likely mortality range for standard spillbays. Muir et al. (2001) found that the estimated relative survival through the Snake River dams was highest at spillbays without flow deflectors (98% to 100%), followed by spill bays with flow deflectors (93% to 100%), bypass systems (65% to 99%), and then turbines (87% to 93%). Ploskey et al. (2001) compiled route-specific survival estimates from PIT-tag studies to characterize total effects (both direct and indirect mortalities) at The Dalles Dam where estimated relative survival of spring migrants was 96%, 92%, and 81-86%, and summer migrant survival was 92%, 93%, and 84% for spillbays, sluiceways, and turbines, respectively. Spill mortality can also vary between dams with and without spill deflectors (Iwamoto et al. 1994; Muir et al. 1995, 1996, 1998, 2001) and with localized hydraulic conditions such as back eddies or islands, that may provide habitat and refuge for predators.

Spill may also increase fish survival by shortening migration times through forebays, tailraces, and reservoirs, and by minimizing exposure to predators, stressful temperatures, and fish pathogens. Spill was shown to be an important factor in reducing forebay residence times in studies conducted by Snelling and Schreck (1994). Studies by the USGS showed that, in general, yearling and subyearling Chinook salmon and steelhead that arrived in the forebay when no spill occurred tended to delay, thus increasing residence times in the forebay of John Day Dam (Hansel et al. 1999, 2003; Beeman et al. 2003). High spill volumes and water velocities can promote the egress of juvenile salmonids and disperse predators from tailrace areas, thus reducing predation risk (Faler et al. 1988; Shively et al. 1996). Spill patterns and flows also influence egress and survival through tailraces by minimizing lateral flow across the dam and directing flow away from predator habitats near islands and shorelines and towards the main channel (Beeman et al. 2003).

Spilling water at dams may also provide system-wide survival benefits. An analysis of juvenile salmon survival in the lower Columbia River index reach in 2001 was evaluated by studying periods of passage at McNary Dam (FPC 2001). A plot of the estimated survival from McNary Dam tailrace to Bonneville Dam tailrace suggested an increase in the estimated survival of yearling Chinook salmon passing McNary Dam that corresponded with the initiation of spill in the lower Columbia River at The Dalles and Bonneville dams on May 16 and John Day Dam on May 25 (FPC 2001, Figure 44). Although this study suggested that spill was a causal mechanism for the trends in survival, the investigators expressed concern that other confounding factors make it difficult to attribute the effects to spill alone. Further, the NMFS 2000 BIOP (Appendix E) projected a 4% to 6% increase in system survival of juvenile salmonids with a managed spill program up to the 120% TDG level. Recent research and biological monitoring results support the findings of the NMFS 1995 report, which predicted that TDG in the 120% to 125% range, coupled with vertical distribution fish passage information indicating that most fish migrate at depths providing some gas compensation, would not cause juvenile or adult salmon mortalities exceeding the expected benefits of spillway passage.

Recently, the efficacy of continuing spill programs later in the migration season (i.e., late-July –August) has been questioned for a variety of reasons that have been discussed in various regional forums. Some have suggested that the majority of endangered Snake River fall Chinook salmon are transported around FCRPS dams in the summer and that few ESA listed fish remain in-river to benefit from a summer spill program. Additionally, since the water spilled to aid passage of juvenile salmonids results in lost power generation, there has also been recent interest in evaluating the cost effectiveness of the spill program, including spill during the latter part of the juvenile migration season (Independent Economic Advisory Board; IEAB). The IEAB used data and models from a variety of sources to provide preliminary examples of the potential costs of various spill scenarios. However, the IEAB acknowledge the preliminary nature of the analyses and the existence of information gaps that, if filled, would improve their analyses.

Few studies examining the passage benefits of spill have been conducted during the latter part of the annual spill program (e.g., August). A balloon-tag survival evaluation conducted at The Dalles Dam in August 2002 suggested that high predation rates on juvenile salmonids might be occurring for spillway-passed fish. Because of these and other issues surrounding the spill program at FCRPS dams, various agencies with disparate views of the costs and benefits of the spill program have requested that additional studies be conducted. In the 2003 Mainstem Amendment to the Fish and Wildlife Program, the Northwest Power and Conservation Council called "for NOAA Fisheries, the federal operation agencies, and salmon managers to immediately implement tests to examine the benefits of the current summer spill program for outmigrating juvenile fall Chinook salmon, and to determine whether the biological benefits can be achieved in a more effective and less costly manner."

### Critical Uncertainties

During 2004, the USGS conducted survival studies at Bonneville Dam designed to estimate the survival of subyearling Chinook salmon during two spill operations. In addition to the assumptions commonly associated with using survival models, there are additional assumptions associated with this particular study because of the specific management objectives being evaluated. These assumptions are a result of either technological (e.g., tag size and battery life) or logistic (e.g., inexperience when conducting radio-telemetry studies during late-July and August and at the temperatures commonly seen during these periods) limitations. As is true with most technological and logistic problems, these limitations will likely be overcome as technological advances are made and efforts are directed at overcoming the logistic difficulties. The underlying assumptions associated with the proposed USGS survival studies at Bonneville Dam during 2004 lead to the following critical uncertainties:

1) Spill treatments affect survival rates for fish < 110 mm in the same way they affect rates for fish >110 mm. That is, the sub-sampled population of fish > 110 mm is representative of the entire population. This assumption is inherent in the proposed approach because the smallest (given recommended tag weight to fish weight ratios) subyearling Chinook salmon that can be radio-tagged is 110 mm given the current technology.

2) The effects of spill on fish passing the dams during the spill treatments between ~ 20 June – 31 July are the same as the effects of spill on fish passing the dams in August.

3) In late July – August, river temperatures are too high to safely handle and tag fish.

To address these and other existing critical uncertainties associated with radio telemetry studies that have the goal of assessing the behavior and survival of juvenile subyearling Chinook salmon during late-July and August, the USGS proposed to conduct a combination of retrospective analyses and laboratory and field experiments. During 2004 and early 2005 we will complete retrospective analysis and pilot studies in the laboratory and field. Additional laboratory and field experiments are proposed for 2005 with analysis being completed in early 2006 (see Schedule in Appendix Table 1).

## Current Status

As of late July, 2004, we have made progress on several tasks scheduled for summer 2004. We believe research activities proposed for summer of 2005 are justified based several of these recent findings. First, based on bench tests, we believe the antenna length of radio tags can be reduced significantly, perhaps as much as 30%. Furthermore, it may be feasible to switch to antenna material that is about 50% of the weight and diameter of the antenna currently used. One concern of this lighter antenna material is the propensity of two antennas to tangle, but tangling of antennas has not been observed in preliminary field tests underway at this time. These changes alone to the antenna configuration of the transmitter could substantially reduce the weight of currently available transmitters.

A decrease in the weight of tags will significantly reduce the number of fish handled to obtain the large-sized fish needed for transmitters currently used to estimate survival of juvenile salmonids. We believe the radio tag weighing 0.85 g that is currently used to estimate survival can be reduced in weight by about 40% to 0.5 g. Furthermore, the 0.5 g tag may be able to be used for survival estimates in summer 2005. This will enable researchers to be less selective for size and select experimental fish from a larger proportion of the population. With a 0.5 g tag we could implant transmitters in fish as small as 95 mm. Subyearling Chinook salmon >95 mm represented over 80% of the population passing McNary Dam between 2000 and 2004. The field tests proposed herein for summer of 2005 will serve as quality control for survival estimates of relatively small Chinook salmon that we have not tagged in the past studies.

Laboratory experiments are currently underway to identify the transmitter technology that minimizes negative effects on subyearling Chinook salmon used in telemetry studies. We believe the activities proposed for 2005 will increase the certainty of findings obtained from these laboratory experiments. For example, we propose to continue some laboratory tests under field conditions at lower Columbia River dams. This strategy has several advantages. First, we will use run-of-the-river fish that will be more representative of the fish being used for survival estimates. The literature is replete with examples of differences in the condition of fish in the hatchery or laboratory and during the migration. Second, the thermal history of fish will be representative of fish used for survival studies. Although we can mimic this history in the laboratory by water temperature control, the preferred experimental animals are the subyearling Chinook salmon at the dams that are normally used to estimate survival.

## Objectives and Methodology

**Objective 2.** Determine the effects of radio-tagging subyearling Chinook salmon at elevated water temperatures.

Rationale:

To evaluate the efficacy of summer spill as a means to pass juvenile fall Chinook salmon over Columbia River dams, radio telemetry studies are being used to estimate survival of fish passing through dams. Although juvenile salmonids in the Columbia Basin are routinely implanted with radio-tags, little is known about the effects of tagging small fish (e.g., less than 110 mm) at high water temperatures (e.g., from 21° to 24°C). Fish that are handled, tagged, and released at high temperatures may have decreased performance relative to those tagged at lower temperatures and to fish that are not tagged, but these hypotheses have not been tested. Therefore, we propose to evaluate mortality, physiological status, and swimming performance of radio-tagged fish at temperatures greater than 21°C using a combination of field and laboratory experiments. Mortality estimates obtained during these experiments will be compared to the observed system mortality at juvenile fish passage facilities as determined by the Smolt Monitoring Program. This program is overseen by the Fish Passage Center. The responses of fish tagged at temperatures > 21°C will be compared to: (1) fish tagged at or slightly below 21°C, which is the maximum temperature that we are currently allowed to tag fish under some Endangered Species Act permits; and (2) fish that are handled and held at temperatures > 21°C but not tagged. Evaluating lethal and sublethal effects of tagging fish at high temperatures is important because only slight excursions of temperature above or below those normally encountered can elicit signs of thermal injury (Logue et al. 1995). Ultimately, the combination of field studies using active migrants and controlled laboratory studies will provide the greatest understanding of the effects of tagging fish at high temperatures.

In July and August of 2004, a pilot study was conducted at The Dalles Dam to evaluate the effects of tagging fish at elevated river temperatures. The work is currently on going, and preliminary results will be available in October 2004. For the pilot study, ambient river temperatures were used, and trials were conducted from mid-July through mid-August in an attempt to capture the greatest possible range of temperatures. For 2005, we propose to return to the field to complete a more rigorous evaluation of the effects of elevated temperature. By March 2005 we will have completed preliminary laboratory experiments (see Task 2.2 from 2004 proposal, and described in Task 1.2, below) and will have some insights into what temperature range causes increased mortality of tagged fish. Using these data, we can target specific temperatures of interest to be tested in the field with actively migrating fish. During the pilot study, we were not able to influence river temperatures, but in 2005, we can use heated river water, if needed, to reach our test temperatures.

**Task 2.1.** Evaluate mortality and physiological responses of radio-tagged subyearling Chinook salmon at temperatures they would experience in the Columbia River in late July and August.

We propose to measure the lethal effects of tagging fish at high temperatures by conducting mortality bioassays at John Day, The Dalles, or Bonneville dams. The 2004 pilot study was conducted at The Dalles Dam, so we propose alternate or additional sites in 2005. We will compare the rate of mortality between tagged and non-tagged (control) fish held for 4 d at ambient river temperatures. This 4 d holding period (over twice the time required for 99% of fish to travel from John Day to The Dalles Dam or from Bonneville Dam to the I-205 bridge) will allow an assessment of short-term and delayed mortality and will minimize any potential deleterious effects of holding actively migrating smolts in tanks for extended periods of time. The null hypotheses addressed by this task include the following:

Ho: "Short (24 h) or long-term (96 h) mortality of fish that have been collected, transported, and held at > 21°C is not greater than that for fish at ≤ 21°C"

Ho: "Short (24 h) or long-term (96 h) mortality of fish that have been collected, transported, held, and tagged at > 21°C is not greater than that for fish at ≤ 21°C"

Ho: "Mortality of fish at temperatures > 21°C attributable to the implantation of radio-tags is not greater than that of fish experiencing temperatures ≤ 21°C"

In our opinion, measuring only mortality rate provides an insufficient evaluation of the effects of tagging fish at elevated temperatures. In short, when evaluating the effects of elevated temperatures on fish, death is ecologically too late. Thus, we will evaluate some sublethal effects of tagging fish at elevated temperatures by measuring key indicators of physiological stress over time. Specifically, we propose to measure levels of plasma cortisol and liver heat shock protein 70 (hsp70) in tagged and non-tagged fish several times during the holding period. Measuring cortisol concentrations will allow us to assess the severity of the complete suite of stressors experienced by fish. Measuring levels of hsp70 in the liver will provide more specific, and perhaps more compelling, insight into the effects of tagging and temperature since elevations of hsp's are indicative of cellular damage that occurs prior to death (Sanders 1993; Iwama et al. 1998). Specifically, the null hypotheses addressed by this work are :

Ho: "There are no differences in physiological responses between fish that have been collected, transported, and held at ≤ 21°C or > 21°C"

Ho: "There are no differences in physiological responses between fish that have been collected, transported, tagged, and held at ≤ 21°C or > 21°C"

Ho: "The physiological responses of fish attributable to the implantation of radio-tags do not differ between fish experiencing temperatures ≤ 21°C and > 21°C"

**Activity 2.1.1.** Evaluate short-term and delayed mortality of actively migrating radio-tagged subyearling Chinook salmon.

Subyearling Chinook salmon will be collected as needed from early July to late August from the John Day or Bonneville Dam smolt monitoring facilities. Mortality bioassays will be conducted using two groups of fish. One group (control fish) will be collected, transported, and held at low densities in tanks or buckets located at the dam. The other group (treatment fish) will be collected, transported, tagged, and held. Treatment fish will be gastrically implanted with radio transmitters (model NTC-3-1, Lotek Wireless Inc.) using procedures similar to those described in Adams et al. (1998a, 1998b). Size requirements for fish will follow the minimums previously used by USGS survival studies (i.e., 110 mm FL, 13 g), and we will use fish of uniform size. Tagged and non-tagged fish will be held at test temperatures for 4 d. Specific temperatures under study will be determined following completion of the 2004 field pilot study and the laboratory studies.

Fish will not be fed during the trials, but will be checked daily to record mortalities and regurgitated tags. We plan to begin trials in July, depending on the test temperature. Trials will begin when river temperatures are somewhat below the test temperature, and water will be heated (as needed) to reach the test temperature. With a 4 d holding period, we anticipate conducting 4 to 6 separate trials of this experiment. After a trial is over, we will release all survivors to the river.

For each temperature trial, we will compare the mortality rate at 24 and 96 h post-tagging between treatment and control fish using Fisher's Exact Test. We will also use logistic regression analysis to evaluate mortality rates of control and treatment fish in the repeated trials over time. Required sample sizes for one-tailed comparisons of tagged and non-tagged fish were calculated based on the binomial distribution, with a power of 0.80 and alpha = 0.05. We assumed a maximum mortality rate of any group to be 25%, which allows us to use fewer fish for each trial and still be able to detect a 15%, or smaller, difference in mortality between treatment and control fish. Thus, for each trial, we will require 64 fish per group. Pending the findings of the 2004 pilot study, we may adjust sample sizes to detect smaller (<15%) differences between groups.

*Schedule*: July–August 2005

**<u>Activity 2.1.2.</u>** Evaluate some physiological stress responses of tagged and untagged fish held for 4 d at elevated temperatures.

This experiment, which will be completely separate from that described in activity 1.1.1, will be conducted during a time when temperatures will range from 19-21°C (around mid-July) and again when river temperatures (or heated river water) reach our test temperature. We will conduct this experiment at the same facility used for activity 1.1.1. To evaluate the physiological responses of fish to tagging at high temperatures, we will create treatment and control groups as described above, stock them in pairs, into buckets, and sample them for tissues at selected times after tagging. We will collect blood, gill, and liver tissue from 10 fish in each group at 0, 1, 6, 12, 24, 72, and 96 h post-tagging. Levels of plasma cortisol, gill $Na^+/K^+$-ATPase (as an indicator of degree of smoltification), and liver hsp70 will be measured using enzyme-linked immunosorbent assays (ELISA's). The total number of fish needed for this experiment is 260. We will calculate the mean and SE for all physiological constituents at each sample period and plot them over time. Within each sample period, we will compare the means of treatment and control fish using two-sample *t*-tests. We will also compare the means at similar time periods between fish tested at the two temperature regimes.

Because the health status of fish can influence levels of plasma cortisol and hsp70, all fish in the physiological experiment will be sampled for levels of *Renibacterium salmoninarum* (Rs), the causative agent of bacterial kidney disease, and *Flexibacter columnaris* (Fc). Kidneys will be removed from all fish and levels of Rs will be determined using an ELISA. To test for the presence of Fc, gill swabs will be taken from each fish and streaked on a Petri dish containing Fc media. Culture plates will be incubated for 3- at 20°C and visually inspected for Fc bacteria. Monitoring Rs and Fc infection levels in each group will allow us to assess the contribution, if any, of pathogens to the physiological responses we observe.
*Schedule*: July–December 2005

**Task 2.2.** Evaluate mortality, and physiological status of hatchery-reared radio-tagged and untagged subyearling Chinook salmon at discrete temperatures in a laboratory setting.

We propose to conduct a series of laboratory experiments to determine more precisely the temperatures at which fish performance becomes compromised. This work will focus on determination of temperature thresholds beyond which tagged fish will experience increased mortality, and physiological dysfunction. Such information, collected under more controlled conditions than those in the field, will be useful for interpretation of results from our field experiments.

At the time of the writing of this proposal, we are preparing to conduct laboratory experiments. Research fish have been acquired and are being reared to the minimum size needed to implant radio transmitters (13 g). Due to the unusual timing for the start of this research (work began in June), we will be conducting laboratory experiments during the fall. The better experimental design would be to conduct laboratory experiments following the natural timing of active migrants (i.e., conducting experiments in July and August). Pending the completion of those experiments, we propose to continue laboratory tests in the summer of 2005 to further investigate temperature effects on tagged and untagged fish.

**Activity 2.2.1**. Determine the mortality rate and stress protein expression for radio-tagged and untagged subyearling Chinook salmon held at discrete temperatures

Subyearling Chinook salmon of uniform size will be obtained from the USFWS Little White Salmon National Fish Hatchery for these tests. Groups of fish will be acclimated for one week to test temperatures. Temperatures of 19, 21, 23, 25, and 27°C will be tested in fall, 2004. Following completion of the fall/winter experiments we may need to more closely define the temperature at which stress effects are most pronounced. The two-degree differential experimental design used for the fall/winter tests may need to be refined by using a one-degree differential. The modified design would then test for temperature effects at 1-degree increments within the 19° to 27°C range; the actual temperature to be selected based on the results of the 2°C-increment tests.

After a one week acclimation period at the test temperature a sample of fish at each temperature will be implanted with dummy radio tags and placed in each of three tanks. Control fish will be sham implanted (i.e., captured, handled, anesthetized, and returned to their tank) and also placed in triplicate tanks. Each tank will be checked at 24, 48, 72, and 96 h post-stocking. For each temperature, replicate tanks within each treatment will be pooled and mortality rates compared using Fisher's Exact Test. We have designed this experiment to detect a 15% difference in mortality rates between treatment and control fish, assuming a maximum expected mortality rate of 25%, alpha = 0.05, and power = 0.80. Thus, for each temperature, each replicate tank will contain 64 fish. To determine the temperature at which hsp70 is expressed significantly beyond background levels, we will place an extra 20 fish in each tank during the mortality bioassays. We will sample 10 fish from each tank at 24 and 96 h, remove their livers, and assay them for expression of hsp70. Mean levels of hsp70 will be compared between treatment and control fish at each temperature using two-sample *t*-tests. Mean levels of hsp70 in all fish will be plotted against temperature to determine the temperature, or temperature range, when hsp70 becomes significantly elevated.

To best simulate the natural photoperiod and physiological status of fish, these laboratory experiments should be conducted during the outmigration period for subyearling Chinook salmon (July-August). We would propose to repeat some of the temperature trials that will be conducted in fall during the summer of 2005. These experiments would be conducted at the same time as the proposed field experiments.

*Schedule*: March – August, 2005

**Objective 4.** Identify the transmitter technology that minimizes negative effects on subyearling Chinook salmon used in telemetry studies.

Rationale:

Due to the small size of sub-yearling Chinook salmon and the size of transmitters currently available, survival estimates obtained using telemetry (radio or acoustic) are limited to larger individuals of the population. A critical uncertainty is whether results from survival studies apply to the entire population or only that fraction of the population represented by larger individuals. To address this uncertainty, researchers should use transmitters that can be implanted in smaller individuals, thereby representing a greater proportion of the population. For subyearling Chinook salmon, this transmitter will have the smallest possible size and physical properties that minimize impacts on fish behavior and survival. One working hypothesis is that acoustic transmitters have fewer negative effects on fish behavior than radio transmitters because acoustic transmitters lack an external antenna. However, there is little empirical data showing a radio tag affects fish behavior more than an acoustic tag. If the antenna negatively impacts fish behavior, shortening the antenna may minimize these effects.

Advancements in the design of both radio and acoustic transmitters may allow tagging of smaller individuals. Current transmitters weigh as little as 0.85 g, but radio and acoustic transmitters weighing 0.5 g will soon be available. In addition, research is underway to develop transmitters that are partially neutrally buoyant, thereby reducing the weight in water that a fish must carry. When choosing among the available technological advancements, two important questions arise: 1) Which transmitter design has the least affect on subyearling Chinook salmon? and 2) What is the minimum size of fish that can be tagged with negligible effects on behavior and performance of subyearling Chinook salmon? Implied here is that the transmitter with the least effect can be implanted in the smallest fish, thereby representing the largest proportion of the population.

To understand which type of transmitter has the least affect on subyearling Chinook salmon, we will compare the performance of fish implanted with three types of transmitters: an acoustic transmitter which lacks an antenna, a radio transmitter with a trailing flexible antenna, and a partially neutrally buoyant transmitter that weighs less in water than conventional transmitters. We will explicitly incorporate fish size as a factor in our experiments to determine the threshold where transmitters begin to affect the performance of tagged subyearling Chinook salmon. Because there has been no consistent guidance on maximum tag ratios or the transmitter technology with the least effect on fish, the trend has been to conduct a laboratory study with smaller fish each time a new transmitter is developed. In contrast, our goal is to provide general criteria that can be applied to different sizes and types of transmitters as they are developed. This information should reduce the need to conduct another laboratory study each time a smaller transmitter of a particular technology is developed.

In 2005, we propose to extend our laboratory studies conducted during 2004 to a field setting to use run-of-the-river subyearling Chinook. We believe this approach will increase the certainty of findings obtained from the laboratory experiments. First, run-of-the-river fish will be more representative of the fish being used for survival estimates. The literature contains many examples of differences in the condition of hatchery/laboratory fish compared to fish in the river during their migration. Second, environmental conditions during field experiments, such as the thermal history of fish, will better represent the population of fish used for survival studies. Although we can mimic this history in the laboratory by water temperature control, the preferred experimental animals are the subyearling Chinook salmon at the dams that are normally used to estimate survival. Last, findings from the laboratory experiments will help to guide and refine experiments conducted in the field on run-of-the-river fish. For example, if laboratory experiments show that the antenna affects fish performance, we will experiment with yet shorter antennas during the field studies. As another example, for the laboratory experiments we chose a tag that was only partially neutrally buoyant because we were concerned that the increased volume of this tag may limit implantation into smaller individuals. If we find the partially neutrally buoyant tag has less of an effect on fish than standard tags, then we will extend the experiments in the field to determine whether it is feasible to implant fish with larger volume tags that weigh even less in water.

**Task 4.1.** Compare the performance of subyearling Chinook salmon implanted with radio tags and acoustic tags and determine the critical fish size at which performance of tagged fish differs from controls.

Treatments - Treatments in this experiment will consist of fish implanted with an acoustic transmitter, fish implanted with a radio transmitter, and a control group which will be handled but not tagged. All fish will be gastrically implanted with transmitters using the methods of Adams et al. (1998a, 1998b). We will use dummy transmitters representing the smallest transmitters that will be available for widespread use in the near future. We anticipate these transmitters will weigh about 0.5 g. Dummy acoustic and radio transmitters will be produced to have equal weights – the only difference between the radio and acoustic transmitters will be the presence of the antenna. Therefore, the body of the radio transmitter will have less mass than the acoustic transmitter since some of the mass will be taken up by the antenna. We will use the shortest antenna length and lightest material as identified from the findings Objective 3 in the 2004 proposal. In addition, the transmitter length may be further altered based on the findings of laboratory experiments conducted in 2004.

Performance metrics – We propose to measure burst swimming speed and swimming orientation of subyearling Chinook salmon implanted with acoustic and radio transmitters. Fish likely use burst swimming (anaerobic swimming at high speeds for short bursts) during dam passage to escape a predator or avoid impingement on screens or other structures. We selected burst swimming as a performance metric to better understand how transmitters affect swimming performance at dams where tagged fish must respond to drastic changes in velocity or an encounter with a predator.

We chose swimming orientation as a second performance metric to compare acoustic and radio transmitters because the antenna of a radio transmitter may affect behavior of tagged fish. Implanting transmitters into fish may change their center of mass and buoyancy causing the fish to alter its swimming orientation. Observations of small tagged fish, below the usual lower size limit, show that fish will "pitch" in a head up position. Furthermore, the drag of an antenna that is longer than the fish may be biologically significant. Anglea et al. (2003) used similar rational as one factor supporting the use of acoustic transmitters, which have no external antenna, in studies of juvenile Chinook salmon.

Fish source, selection, and sample size - Experiments will be conducted at one of the Lower Columbia Dams (to be determined) using run-of-the river subyearling Chinook salmon. To incorporate size as a factor, we will select fish based on stratified random sampling where equal numbers of fish in specific size groups will be randomly assigned to each treatment. The range of fish size will represent tag ratios between 2% and 10%. Ten fish will be selected for each 1% increment in tag ratio between 2% and 10%, yielding a sample size of 80 fish per treatment and a total of 240 fish for each performance metric. This sample size is based on the work of Nelson et al. (2002) and should yield sufficient statistical power ($1-\beta \geq 0.80$) to detect a relationship between fish size and performance. However, we will refine these sample sizes by conducting a power analysis from the findings of the laboratory experiments conducted in 2004.

Experimental procedures – Burst swimming performance will be measured with a laser diode/photocell timed 'drag strip' swimming chamber (Nelson et al. 2002). The swimming chamber consists of an acclimation chamber on the downstream end with a 2-m long, narrow channel on the upstream end (the "drag strip"). Spaced along the narrow channel are six lasers and light detectors. Fish will be held in the acclimation chamber for 24 hours with a gate over the narrow channel. After the acclimation period, the gate will be raised and the fish startled causing it to burst down the narrow channel. As the fish passes through the channel, a computer will record the time that the fish passes through each laser beam. From the known distance of each laser and the time to pass each laser, the swimming speed and acceleration of the fish can be calculated. Burst swimming performance of tagged and untagged fish will be evaluated by examining swimming speed and acceleration as a function of elapsed time and distance. In addition, maximum swimming speed ($V_{max}$) will be compared among the treatment and control groups.

The effect of the radio and acoustic tags on fish orientation will be evaluated by measuring the angle of the fish's longitudinal axis from the horizontal. Fish swimming orientation will be measured at a range of water velocities to examine how water velocity affects the orientation of tagged and untagged fish. The testing apparatus is a large swimming tunnel approximately 0.6 m long x 0.5 m high x 0.5 m wide. Five fish will be placed in the test chamber and allowed 4 hours to acclimate. At the start of the test, fish swimming behavior will be recorded using a video camera beginning at a velocity of 1 body length/s and incremented by 0.5 body length/s every 15 min up to when the fish does not maintain position in the test chamber. The video recording will be analyzed by randomly selecting 10 frames from each velocity increment and measuring angle of the longitudinal axis of the fish from the horizontal. The initial buoyancy of fish and their depth in the swimming tunnel may affect swimming orientation. Therefore, as auxiliary variables, we will measure the fish's depth in swimming tunnel. In addition, immediately following testing of fish, each fish's buoyancy will be measured in a series of graded salinity baths following the methods of Perry et al. (2001).

<u>Hypotheses and data analysis</u> – Several hypotheses can be tested using this experimental design:

1) $H_0$: There is no difference among treatments (acoustic vs. radio, radio vs. control, or acoustic vs. control) in the burst swimming speed or swimming orientation of subyearling Chinook salmon.
2) $H_0$: No relationship exists between fish size and burst swimming or fish size and swimming orientation.

These hypotheses will be tested using an analysis of covariance with burst swimming speed and swimming orientation as the dependent variables. Factors used in the analysis will be treatment (test of hypothesis 1), fish size (test of hypothesis 2), and the interaction term and treatment x size. If we find that fish size affects the performance of tagged fish compared to controls, we will use broken-stick regression models to estimate the threshold tag ratio at which performance is compromised.

<u>Expected products</u> – Our experiments will identify the critical fish size at which each type of transmitter (radio and acoustic) affects fish performance. This minimum fish size will also be expressed as the maximum tag ratio, which will provide a general guideline in the future as technological advances further reduce transmitter size. Our experiments will also identify whether transmitters with antennas affect fish performance more than transmitters without antennas. These field experiments with run-of-the river fish will help to validate and confirm findings conducted in the laboratory on hatchery-reared fish.

**Task 4.2.** Determine whether the weight of transmitters in water or in air is the limiting factor that compromises performance of relatively small subyearling Chinook salmon.

Treatments – The treatments will include: 1) fish implanted with a tag with a weight in air 0.5 g and a weight in water of about 0.3 g, 2) fish implanted with a tag of the same weight in air but half the weight in water, and 3) control fish which will be handled but not tagged. These two tags weigh the same in air, but the main difference is that one tag will have a larger volume due to encapsulated air that will reduce its weight in water. We chose a tag with half the weight in water of a conventional tag because we believe a completely neutrally buoyant tag may be of too great a volume to be practical for use on relatively small subyearling Chinook salmon. However, based on findings of the laboratory experiments conducted in 2004, we may extend these experiments to test larger volume tags that weigh even less in water. Both tags will lack an antenna to eliminate any possible confounding effects of the antenna on fish performance. All fish will be gastrically implanted with tags using the methods of Adams et al. (1998a, 1998b).

Performance metrics – We propose to measure fin beat rate, swimming orientation, and buoyancy of subyearling Chinook salmon at range of pressures that will simulate a range of depths. We will estimate performance metrics at a range of pressures to better understand how tagged fish behave as they sound and approach deep passage routes at dams. Coutant and Whitney (2000) suggested that buoyancy of fish passing through turbines could be important in determining where fish pass through turbines and their vertical orientation during turbine passage, both of which may affect the likelihood of striking turbine runners. The proposed performance metrics should change with pressure because pressure compresses a fish's airbladder, decreasing buoyancy (Alexander 1966), and fish respond to decreases in buoyancy by increasing compensatory swimming movements and changing their swimming posture. For example, Harvey (1963) found that as pressure was increased, juvenile salmon angled upward towards the surface and increased their rate of pectoral fin beats to compensate for negative buoyancy. Perry et al. (2001) showed that the buoyancy of tagged fish changes at a higher rate in response to pressure than that of untagged fish. These findings suggest that tagged fish may expend more energy and have a different swimming posture than untagged fish at the same depth. A partially neutrally buoyant tag could offset these effects, but has not yet been compared to conventional transmitters in laboratory experiments.

Fish source, selection, and sample size – Experiments will be conducted at one of the Lower Columbia Dams (to be determined) using run-of-the river subyearling Chinook salmon. In contrast to Task 4.1, fish size will be constrained to represent a small range of tag ratios between 5% and 7% (the maximum tag ratio currently used in field studies) to control for the effect of fish size on performance metrics. Since performance will be measured over a range of pressure, we will minimize the range in fish sizes to limit the number of potential covariates that may affect the performance measures. For a 0.65 g tag, for example, tag ratios between 5% and 7% represent fish weighing between 9 and 13 g. Within this size range, 50 fish will be randomly assigned to each of the three treatments, for a total sample size of 150 fish. This sample size is based on results of Perry et al. (2001) and Harvey (1963) and should yield sufficient statistical power ($1-\beta \geq 0.80$) to detect a difference between treatments. However, we will refine these sample sizes by conducting a power analysis from the findings of the laboratory experiments conducted in 2004.

Experimental procedures – Fish will be introduced to a pressure chamber and allowed to acclimate for 1 h. To start the test, swimming behavior of fish will be recorded with a video camera at atmospheric pressure and then incremented by 0.25 atmospheres every 10 min up to a pressure of 2 atmospheres (equivalent to a depth of 20 m; 1 atmosphere = 760 mm Hg). The video data will be analyzed at each pressure increment to measure pectoral fin and tail beat frequency (fin beats/min), the angle of the fish's longitudinal axis from horizontal, and the depth of the fish in the pressure chamber. Immediately following testing of fish in the pressure chamber, each fish will be anaesthetized and its buoyancy measured at atmospheric pressure in a series of graded salinity baths using the methods of Perry et al. (2001). The buoyancy of fish at each pressure increment will be back-calculated by applying Boyle's Law to the buoyancy of fish at atmospheric pressure.

Hypotheses and data analysis- $H_0$: Performance (i.e., fin beat rate, orientation, buoyancy) of subyearling Chinook salmon does not differ among fish without transmitters, fish implanted with conventional transmitters, and fish implanted with transmitters having a reduced weight in water.

This hypotheses will be tested using an analysis of covariance with fin beat rate, swimming orientation, and buoyancy as the dependent variables. Factors used in the analysis will be treatment, pressure, and the interaction term of treatment x pressure. If the weight of the tag in water affects fish more than its weight in air, we would expect to find differences in the relationship of performance metrics with pressure.

Expected products – The tags used in this experiment will reveal whether a tag weighing less in water reduces the affect of the transmitter on fish behavior and performance. In addition, some investigators have suggested using as an index of the tag's weight in water relative to fish weight in air is more appropriate, than weight in air, because it represents the additional mass that a fish must carry (Brown et al. 1999, Perry et al. 2001, Jepsen et al. 2002). Our experiments should identify the most appropriate index to use when considering the impact of a transmitter on small juvenile salmonids. If more neutrally buoyant transmitters minimize tag effects, then it may warrant adding such requirements to specifications on future tag purchases. These field experiments with run-of-the river fish will help to validate and confirm findings conducted in the laboratory on hatchery-reared fish.

# FACILITIES AND EQUIPMENT

The USGS operates the Columbia River Research Laboratory, which includes research boats, vehicles, office space, and laboratory facilities for the conduct of this study. Boats will be operated at cost with no additional lease cost to the project. Boats will be operated by Department of Interior certified boat operators that are trained in CPR and First Aid. To meet U.S. Coast Guard standards, boats will be inspected by a third party. USGS will provide a quality control system consistent with the Good Laboratory Practices Act.

Investment in additional capital equipment is minimal because most equipment needed for the proposed project is currently available at the U.S. Geological Survey's Laboratory. Included in the budget is a request for $5,000 for an additional, appropriately-sized Blazka-style swim tunnel to accomplish Objective 2. Other equipment such as water temperature controllers, swim orientation chamber, laser drag strips burst swimming chamber, and video recording equipment (Objective 4) may require minor modification at the expense of the project.

Other resources include:

-A selection of 25 boats up to 30 feet in length for work on the river.
-Two 2,700 square foot storage facilities with a shop.
-4,000 square foot wet lab facility.
-A local computer network integrating state-of-the-art GIS capabilities.
-A technical staff of 60-100 fishery biologists, ecologists, and GIS specialists.
-An office and analytical laboratory in a 15,000 square foot facility.

# IMPACTS

<u>Impacts to other researchers</u>-We do not foresee any impacts to other researchers as a result of implementation of this study.

<u>Impacts to COE Hydroelectric Project</u>-The activities outlined in this proposal will have minimal impact to the Hydroelectric Projects. We will require some additional coordination to accommodate some of the field studies at the projects but we feel this will be a very minimal impact. The majority of the activities outlined in this proposal will be conducted at our research facility.

# COLLABORATIVE ARRANGEMENTS AND SUB-CONTRACTS

None.

# LIST OF KEY PERSONNEL AND PROJECT DUTIES

| | |
|---|---|
| Noah S. Adams | Principle Investigator – relation to survival studies, tagging protocols |
| John W. Beeman | Principle Investigator – tag design specifications |
| Theresa L. Liedtke | Principle Investigator – conduct of mortality and temperature experiments |
| Matthew G. Mesa | Principle Investigator – physiological assays |
| Russell W. Perry. | Principle Investigator - conduct of tag size, weight, configuration experiments |
| Dennis W. Rondorf | Project Leader – liason with USACE, review of products |
| Alec G. Maule | Project Leader – Supervisory Research Physiologist review of products |

# TECHNOLOGY TRANSFER

We plan to transfer information obtained from our analysis in the manners listed below. Once this information is transferred, it will be used in making decisions relative to operation of the Federal Columbia River Power System and Juvenile Transportation Program. In addition, the information will be used by other federal and state agencies, Indian tribes, and the public to make management decisions to aid in the recovery of threatened and endangered salmon populations in the Columbia Basin.

1. Preliminary reports to the Army Corps of Engineers. A preliminary report of our findings will be submitted on March 31, 2005.

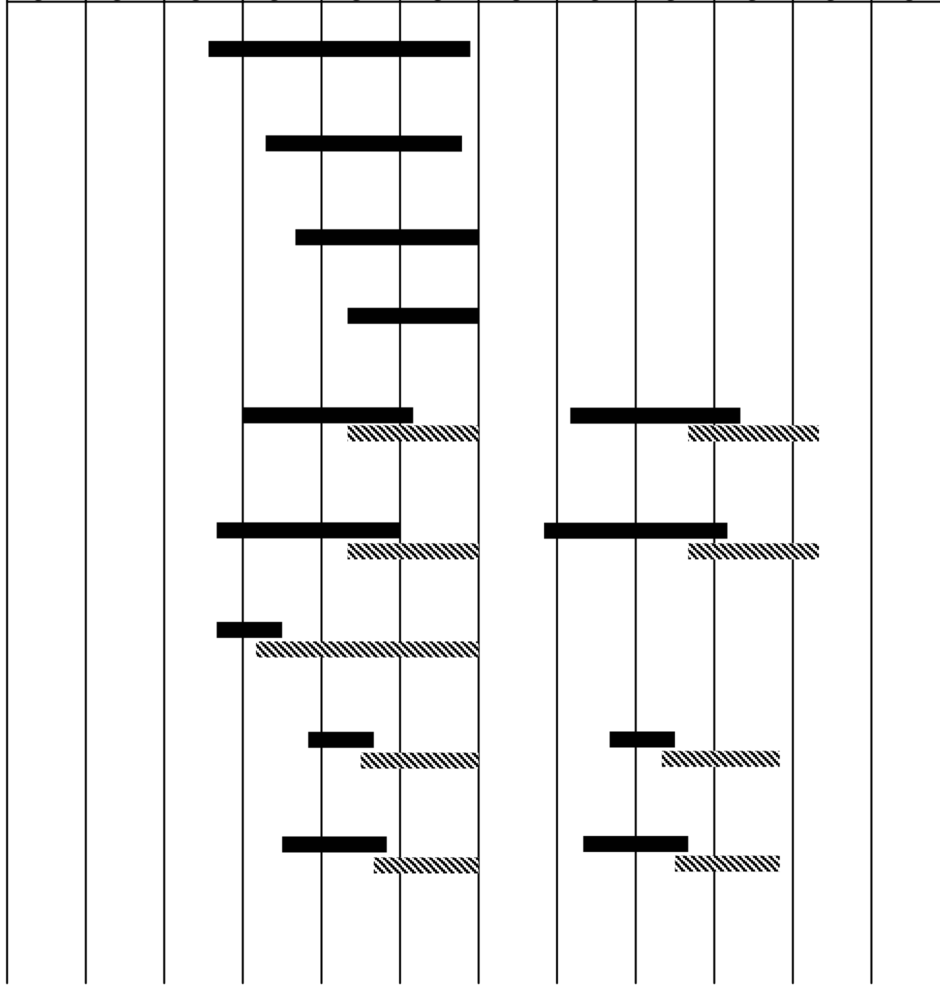
2. Presentation of data obtained to date to fisheries agencies, tribes, and the public at the Anadromous Fish Evaluation Program (AFEP) Annual Research Review, 2004.

3. Presentations to the Army Corps of Engineers staff and study review groups as requested.

4. Presentations at professional meetings (i.e., Transactions of the American Fisheries Society) and publication of information in peer reviewed journals.

Appendix Table 1. Time table showing the time period when tasks are expected to be conducted (dark line indicated experimental period and hashed lines indicate analysis periods.

| Task | Time | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2004 | | | | 2005 | | | | 2006 | | | |
| | Qt 1 | Qt 2 | Qt 3 | Qt 4 | Qt 1 | Qt 2 | Qt 3 | Qt 4 | Qt 1 | Qt 2 | Qt 3 | Qt 4 |
| **Task 1.1.** Recode data to represent fish <120 mm and > 120 mm. | | | | | | | | | | | | |
| **Task 1.2.** Formulate detection histories for recoded data. | | | | | | | | | | | | |
| **Task 1.3.** Produce model results. | | | | | | | | | | | | |
| **Task 1.4.** Prepare preliminary and final reports. | | | | | | | | | | | | |
| **Task 2.1.** Evaluate mortality and physiology of fish at temperatures they would experience in late July and August. | | | | | | | | | | | | |
| **Task 2.2.** Evaluate mortality, physiological status, and swimming performance of fish at discrete temperatures in a laboratory setting. | | | | | | | | | | | | |
| **Task 3.1.** Determine the relation between transmitter antenna configuration and transmitter power. | | | | | | | | | | | | |
| **Task 4.1.** Determine the role of the weight of tags in water and the radio tag antenna in limiting the tagging of small fish. | | | | | | | | | | | | |
| **Task 4.2.** Determine the lower size limit for tagging subyearling Chinook salmon. | | | | | | | | | | | | |



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